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Separating esterase targets of organophosphorus compounds in the brain by preparative chromatography

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HIGHLIGHTS

- Highly sensitive soluble carboxilesterases to OPs have been found in chicken brain.
- A fractionation procedure was developed by HPLC for chicken brain soluble esterases.
- The preincubated sample with mipafox has distinct ion exchange chromatography profile.
- This change suggests that mipafox modifies the ionic properties of numerous proteins.

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ABSTRACT

Low level exposure to organophosphorus esters (OPs) may cause long-term neurological effects and affect specific cognition domains in experimental animals and humans. Action on known targets cannot explain most of these effects by. Soluble carboxylesterases (EC 3.1.1.1) of chicken brain have been kinetically discriminated using paraoxon, mipafox and phenylmethyl sulfonylfluoride as inhibitors and phenyl valerate as a substrate. Three different enzymatic components were discriminated and called Eα, Eβ and Eγ. In this work, a fractionation procedure with various steps was developed using protein native separation methods by preparative HPLC. Gel permeation chromatography followed by ion exchange chromatography allowed enriched fractions with different kinetic behaviors. The soluble chicken brain fraction was fractionated, while total esterase activity, proteins and enzymatic components Eα, Eβ and Eγ were monitored in each subfraction. After the analysis, 13 fractions were pooled and conserved. Preincubation of the soluble chicken brain fraction of with the organophosphorus mipafox gave rise to a major change in the ion exchange chromatography profile, but not in the molecular exchanged chromatography profile, which suggest that mipafox permanently modifies the ionic properties of numerous proteins.

1. Introduction

1.1. Organophosphorus compounds (OPs) and unknown neurotoxicological targets

Organophosphorus compounds (OPs) have been used for several purposes, but mainly as pesticides and warfare agents. However, the amount of OPs pesticides being used is declining, especially in developed countries. Notwithstanding, OPs continue to be one of the most important classes of insecticides today (Casida and Durkin, 2013). Indeed it has been reported that 96% of individuals in the US have measurable levels of OPs chlorpyrifos metabolites in their urine (Barr et al., 2004). These compounds can cause several neurotoxic disorders, some of them with molecular identified targets (the cholinergic crisis, the intermediate syndrome and OPIDN), and others with no molecular targets identified to date, whose mechanisms are not well understood (neurobehavioral and cognition long-term toxicity, chronic neuropsychological effects, potentiation of OPIDN, etc.; Roldan-Tapia et al., 2005; Jamal et al., 2002; COT report, 1999; Ray and Richards, 2001).

Acute cholinergic toxicity is caused by covalent organophosphorylation at the acetylcholinesterase catalytic center. Organophosphate-induced delayed neuropathy (OPIDN) is caused by the inhibition and subsequent aging (dealkylation) of the membrane protein called neuropathy target esterase (NTE; Glynn, 1999, 2000). The intermediate syndrome is likely to be a delayed effect of acetylcholinesterase inhibition and acetylcholine accumulation.

However, an increasing number of epidemiological and animal studies have associated subtle, long term central nervous system neurotoxicity (neurobehavioral, cognitive and neuropsychological) consequences – with past overt OP, which known targets cannot explain (Brown and Brix, 1998; Parrón et al., 2011; Roldan-Tapia

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et al., 2005; Yokoyama et al., 1998; Hoffman et al., 2007; Liu et al., 1999). Despite the number of epidemiological studies and several reviews on this topic having been published, the authors have reached conflicting conclusions, which is probably due to different study designs. Ross and colleagues (2013) performed a meta-analysis to quantify and evaluate the data published in 14 studies. This work includes more than 1600 participants who were occupationally exposed to long-term low-levels of OPs, defined as repeated or prolonged exposure to doses that do not produce recognized clinical symptoms of acute toxicity. They concluded that there was sufficient evidence to accept a significant association between low-level exposure to OPs and impaired neurobehavioural function. However, the exact exposure conditions if these effects existed at doses lower than cholinesterase inhibition and if the people had previously suffered an episode of higher or acute exposure, did not come over clearly. The need to investigate how specific low-level OPs affect certain cognition domains and the neurobiological substrates of these effects has been emphasized (Casida and Quistad, 2004). Ray and Richards (2001) reviewed data until 2001. They proposed that any chronic effects of low-level exposures would likely occur via a mechanism that is independent of AChE inhibition.

Potentiation of neuropathy is a neurotoxic effect caused by some esterase inhibitors, not only OPs, but also some carbamates and sulfonylfluorides (e.g., PMSF), when potentiators are administered to hens in conjunction with OPs causing OPIDN, which enhances neurotoxicity (Pope and Padilla, 1990; Lotti et al., 1991). Despite all the efforts made neither the mechanism nor the target of potentiation are known. It has been reported that, given the chemical nature of promoters, the target has to be a similar esterase to NTE, and that is likely to hydrolyse the same substrate (Moretto et al., 1994; Céspedes et al., 1997).

1.2. Potential unknown molecular targets of OPs toxicity

Many enzyme systems have the potential to interact with specific OPs, and the possibility of noncholinergic, non-neuropathic targets existing has been elucidated by various approaches: (1) AChE knockout mice display no AChE activity in any tissue and are sensitive to OP toxicity (Lockdridge and Schopfer, 2006); (2) The secondary target effects that are not specific of AChE inhibitors are observed in zebra fish (Behra et al., 2004); (3) Different OP pesticides cause varying degrees of toxicity despite similar levels of AChE inhibition (Pope, 1999); (4) There is no correlation between AChE inhibition and the disposition of [3H]-soman, [3H]-DFP and [3H]-sarin in the brain (Little et al., 1988); (5) Low doses of OP inhibitors produce distinct effects that depend on the identity of the OP (Moser, 1995); (6) Low levels of chlorpyrifos impair the cognitive function without significantly inhibiting AChE activity and without down regulating cholinergic receptors in rats (Jett et al., 2001) and (7) Biotinylated OP FP-biotin labels at least 12 proteins in mouse plasma at doses that have no cholinergic effects (Peeples et al., 2005).In addition to the known cholinesterases and NTE, others proteins have been observed to covalently bind to OP in experimental assays and in vitro experiments: other serine esterases (KIAA1363 protein, monoacylglycerol lipase, fatty acid amide hydrolase, kynurenine formamidase, etc.), muscarinic receptors and other unknown targets (Casida and Quistad, 2004; Nomura et al., 2008; Nomura and Casida, 2011; Richards et al., 1999, 2000; Murray et al., 2003, 2005). Most of the molecular targets of OPs identified to date are esterases, and elucidate the nature and functional significance of all the OP-sensitive pool of esterases in the central nervous system in order to find novel toxicologically relevant target proteins is an important research task.

1.3. Unknown OPs-sensitive esterases in nerve tissues

Kinetic models have been developed and applied to identify OP-binding enzymes in complex biological preparations. These models consider multi-enzymatic systems with inhibition, spontaneous reactivation, chemical hydrolysis of inhibitor and ongoing inhibition or inhibition during the substrate reaction time. The enzymatic components among esterases in nerve tissues of adult chicken have been discriminated using these kinetic approaches and esterases, which are highly sensitive to OPs, and have been described in soluble fractions of chicken peripheral nerve, chicken brain and chicken serum (Barril et al., 1999; Garcia-Pérez et al., 2003; Estévez et al., 2004, 2010, 2011, 2012; Estévez and Vilanova, 2009; Mangas et al., 2011, 2012), and in the chicken brain membrane fraction (Mangas et al., 2012a,b). It has been suggested that these esterases may play potential roles in toxicity and/or detoxication during low-dose long-term exposure to organophosphorus compounds, which warrants further research.

Special attention has been paid to the phenyl valerate esterases of the soluble chicken brain fractions interacting with OPs (Mangas et al., 2011, 2012b; Estévez et al., 2013). Three enzymatic components have been discriminated with mipafox (an inducer of OPIDN), paraoxon (a non-inducer of OPIDN), and PMSF (an inhibitor model of OPIDN potentiation). These esterase components have been called: Eα, which is highly sensitive to mipafox and paraoxon, but resistant to PMSF, and is spontaneously reactivated when preinhibited with paraoxon; Eβ, which is sensitive to paraoxon and PMSF, but resistant to mipafox; Eγ, which is resistant to paraoxon, sensitive to mipafox and PMSF, which is the fraction that has been named “soluble NTE” or S-NTE (Vilanova et al., 1990). The high sensitivity of Eα to paraoxon and mipafox suggests that it might play a role in toxicity in the low-level long-term exposure of organophosphate compounds, and that it may be relevant only in chronic exposure as it is spontaneously reactivated after paraoxon inhibition. In components Eα and Eγ, exposure to an esterase inhibitor has been seen to modify sensitivity to further exposure to others without any interaction with the hydrolysis of the substrate. Eα becomes less sensitive to PMSF when the preparation is pretreated with mipafox (Mangas et al., 2012b), and is less sensitive to mipafox or to paraoxon after pre-exposure to PMSF (Estévez et al., 2013). Moreover, component Eα loses its spontaneous reactivation capability after pre-exposure to PMSF. The I50 (30 min) of component Eγ to mipafox increases with the PMSF concentration used in pre-incubation, while Eγ becomes less sensitive to mipafox or paraoxon after pre-incubation with PMSF (Mangas et al., 2012a; Estévez et al., 2013). It has been suggested that such interactions might be related to the potentiation of the OPIDN effect (Mangas et al., 2012b; Estévez et al., 2013). A simple method using two mipafox concentrations to discriminate these three components has been proposed (Fig. 1; Mangas et al., 2011).

1.4. Aims

In this work, esterase components that are sensitive to OPs in soluble chicken brain fraction have been fractionated using various preparative high performance chromatography steps. The distribution of the different enzymatic components has been studied in the several fractions obtained. This procedure has concentrated on the proteins in the soluble chicken brain fraction that interact with OPs. Enriched samples have been obtained with enzymatic activities of toxicological interest. The protein profile after inhibition with mipafox has been studied, while the ion exchanged chromatography profile of proteins after mipafox treatment has revealed that numerous proteins peaks changed.